Brief Overview about Oxidative stress in Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an inflammatory and neurodegenerative disorder of the central nervous system (CNS), characterized by immune-mediated damage to cells in the white and gray matter of the brain, spinal cord, and optic nerve through demyelination and axonal degeneration. Oxidative stress is an imbalance between oxidants and anti- oxidants infavor of the oxidants, leading to a disruption of redox (oxidation reduction) signaling and control and or molecular damage. The ROS are reactive species derived from oxygen that include the superoxide anion (O-), hydrogen peroxide (H2O2), and the hydroxyl radical (OH). The RNS are reactive species derived from nitrogen and include nitric oxide (NO·) and peroxynitrite (ONOO-). The ROS and RNS are extremely unstable and reactive because they have an unpaired electron in their outer orbital. They take electrons from proteins, lipids, carbohydrates, and nucleic acids, causing damage to biological membranes, genetic material, and other macromolecules. Oxidative stress is induced by externally added oxidants and compounds either stimulating ROS production or weakening antioxidant defense. The development of neurodegeneration in MS is a complex process that may be related to primary apoptosis, synaptopathy, and excitotoxicity associated with glutamate overload, ionic channel dysfunction, calcium overload, mitochondriopathy, proteolytic enzyme production, and activation of apoptotic pathways. It is also important that mitochondrial dysfunction results in an increased production of reactive oxygen species (ROS), which is detrimental to neurons and glia. On the other hand, OS damages the mitochondria, which disrupts the transport of adenosine triphosphate along the axon, and consequently leads to neurodegeneration.

Keywords: Oxidative stress, Multiple Sclerosis

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Introduction :

Multiple sclerosis is a chronic inflammatory or autoimmune neurodegenerative multifactorial disorder of the CNS; however oxidative stress may be one of the sources or a consequence of the disease from loss of oxidant/antioxidant balance **(1).**

Oxidative stress is an imbalance between oxidants and anti- oxidants infavor of the oxidants, leading to a disruption of redox (oxidation reduction) signaling and control and or molecular damage **(2).**

The ROS are reactive species derived from oxygen that include the superoxide anion (O-), hydrogen peroxide (H2O2), and the hydroxyl radical (•OH). The RNS are reactive species derived from nitrogen and include nitric oxide (NO·) and peroxynitrite (ONOO-). The ROS and RNS are extremely unstable and reactive because they have an unpaired electron in their outer orbital. They take electrons from proteins, lipids, carbohydrates, and nucleic acids, causing damage to biological membranes, genetic material, and other macromolecules **(3).**

The control of ROS steady-state level is provided via their production and elimination, Living organisms possess multilevel and complicated antioxidant system operating either to eliminate ROS or minimize their negative effects. Antioxidants are placed in two groups: low molecularmass antioxidants (usually with molecular masses below one kilo- dalton) and high molecular mass antioxidants (with molecular mass higher than one or actually higher than ten kilodalton). The group of low molecular mass antioxidants includes chemically different compounds such as vitamins C (ascorbic acid) and E (tocopherol), carotenoids, anthocyanins, polyphenols, and uric acid. Most of them are received by human organism as food or supplement components. High molecular weight antioxidants, named macromolecular antioxidants (MA). They are high molecular weight proanthocyanidins and tannins and polymeric structures of low molecular weight polyphenols and carotenoids bound to polysaccharides and proteins found in foods and botanicals **(4).**

Oxidative stress is induced by externally added oxidants and compounds either stimulating ROS production or weakening antioxidant defense. Although it could be expected that increase of external level of oxygen causes oxidative stress with potential tissue injury **and** ischemia/reperfusion might affect biological systems similarly, hypoxia-induced oxidative stress was somewhat unexpected but well experimentally supported to date**(5).**

Under normal conditions, ROS level fluctuates in certain range. Due to some reasons, such as introduction of certain oxidants, ROS level may sharply increase and leave the range of control (rest) conditions. If antioxidant systems are capable adequately cope with enhanced ROS amounts, this level would return into initial corridor. These events may be called ‘‘acute oxidative stress’’that it is not enough to have enhanced ROS level for certain period of time to develop oxidative stress due to expression of antioxidant and related enzymes like superoxide-dismutase (SOD), catalase,

glutathione reductase, etc. (5).In some cases, the cell cannot neutralize enhanced ROS amounts and return ROS level into initial corridor even enhanced expression of antioxidant and related enzymes would not be able to do that. Due to that increased ROS level can be stabilized and enhance modification of different cellular components, substantially disturbing homeostasis, This state can be called ‘‘chronic oxidative stress’’. Finally, one more scenario may take place after oxidative boots or due to change in physiological state of organisms – ROS level may not return into initial corridor and stabilize at new, so-called ‘‘quasi-stationary level’’ (5).

Pathogenesis of Oxidative stress in MS:

The CNS is very active in oxidative metabolism, as it is constantly exposed to low-to-moderate levels of ROS since it has a very active mitochondrial metabolism, which leads to high levels of intracellular superoxide anions, Moreover, oligodendrocytes have low levels of antioxidant enzymes and a high concentration of iron. The ROS are generated by a number of cellular oxidative and metabolic processes including activity of the enzymes of the mitochondrial respiratory chain, xanthine oxidase, NADPH oxidase, monoamine oxidases, and metabolism of arachidonic acid (AA) mediated by the activity of lipoxygenases (LOX), activated macrophages and monocyte-endothelium interactions responsible for immune mechanisms such as phagocytosis. Unsaturated fatty acids are the most vulnerable to free radicals and because myelin has a high lipid-to-protein ratio, it is a preferred target of ROS. In oxidation-reduction balance, the oxidant and antioxidant molecules are in equilibrium, An increase in free radicals results in increased activity of the antioxidant systems, resulting in redox homeostasis. These antioxidant molecules as melatonin, vitamin D, vitamin E, glutathione and antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, etc.) (4).

In chronic inflammatory diseases, such as MS, antioxidant defenses are overcome, which leads to oxidative stress. ROS enhance both monocyte adhesion and migration across brain endothelial cells, Peroxidation of membrane lipids results in the generation of oxidized phospholipids (Ox-PL) and reactive aldehydes which increase blood–brain barrier permeability and induce monocyte binding to endothelial cells. In addition, ROS can activate the expression of the nuclear transcription factor-kappa, which upregulates the expression of many genes involved in MS, such as tumor necrosis factor-α and nitric oxide synthase, leading to mitochondrial dysfunction and energy deficits that result in mitochondrial and cellular calcium overload. Loss of mitochondrial membrane potential can increase the release of cytochrome c, one pathway that leads to neuronal apoptosis. Accumulation of oxidized lipids of myelin and axons and oxidized nuclear DNA of oligodendrocytes (8-hydroxy-D-guanosine) in brain tissue of MS patients leading to demyelination, oligodendrocyte apoptosis and astrocyte dysfunction (6).

There is increase in nitrite/nitrate levels and the activity of Glutathione peroxidase in patients with RRMS compared to healthy individuals. ROS is also considred a marker of oxidative stress in MS

as myelin cholesterol breaks down to 7-ketocholesterol, whose levels in the CSF of MS patients have been reported to be elevated **(7).**

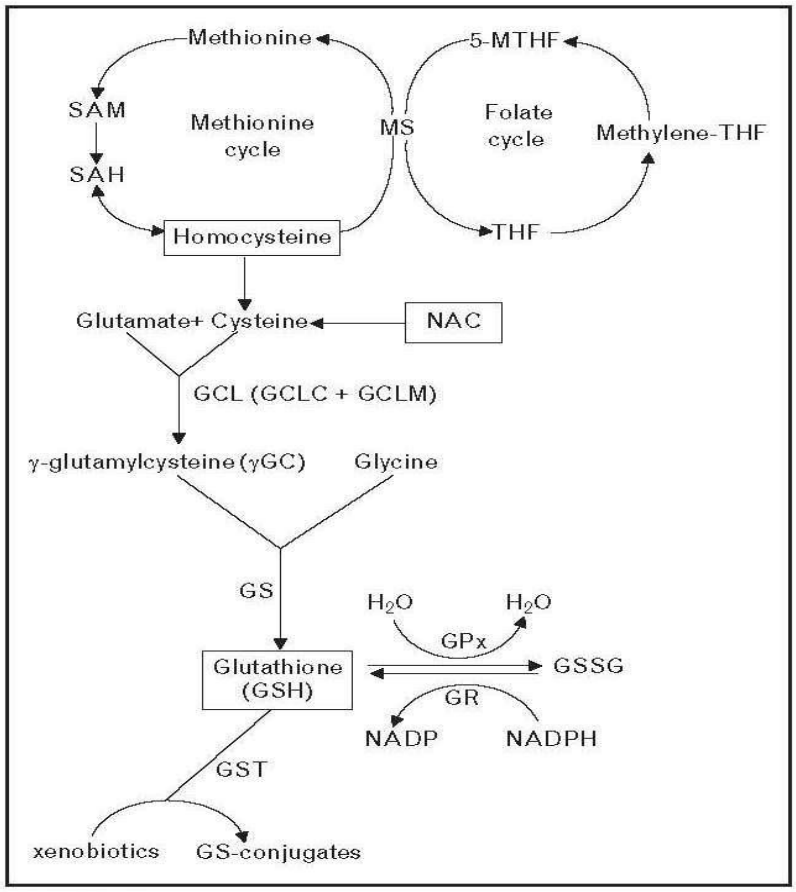
Nitric oxide (NO) and its metabolites can cause mitochondrial damage and tissue hypoxia leading to further damage in MS lesions. High serum and CSF levels of NO were reported in inflammatory neurological disorders. Higher CSF concentrations were further correlated with higher disability progression rates in MS **(8).**

Glutathione (GSH) is the most important endogenous antioxidant and plays an important role in the detoxification of xenobiotics and their metabolites, as well as in the maintenance of the intracellular redox balance which is very important in the brain, where high oxygen consumption produces many harmful free radicals such as ROS. In addition, GSH has other pivotal functions in the cells, such as modulation of cellular differentiation, proliferation and apoptosis. GPx protects the body from oxidative damage by catalysing the reduction of lipid hydroperoxides to their corresponding alcohols, as well as the reduction of hydrogen peroxide to water **(8)**

Glutathione is a tripeptide containing glutamate, cysteine, and glycine amino acids. It is distributed ubiquitously with varying levels throughout the human body. In the brain, GSH concentration is highest in glial cells of the cortex **(9).**

The pathway of maintaining intracellular GSH homeostasis includes GSH redox cycling, direct uptake and de-novo synthesis. GSH is synthesized in the cytosol through two consecutive ATP-dependent enzymatic reactions catalyzed by glutamate cysteine ligase (GCL) and glutathione synthetase (GS). GCL consisting of a catalytic (heavy) subunit (GCLC) and a modulatory (light) subunit (GCLM) mediates the first step of GSH synthesis, that is, the reaction of glutamate and cysteine to form glutamylcysteine (gGC). Then, gGC is coupled with glycine to form GSH in a reaction catalyzed by GS. Cysteine is the rate-limiting amino acid for GSH. **(9).**

synthesis because its levels are lower than those of glutamate or glycine, and GCL is the rate-limiting enzyme. The sulfhydryl group of the cysteine moiety provides the reducing equivalents of the GSH **(10).**



(10).

Reduced glutathione (GSH-g-glutamyl-cysteinyl-glycine) is one of the most important agents of the endogenous antioxidant defence system and protects cells against damage resulting from exposure to agents such as iron, radiation and free radicals, providing protection against oxidative stress. In particular, GSH provides the first line of defence against singlet oxygen and hydroxyl radicals, which are known to cause damage and cell death by apoptosis and necrosis (11).

Glutathione peroxidase catalyses the reduction of hydroperoxides sush as H2O2 to oxidised glutathione (GSSG) to protect cells against oxidative damage and GSSG can also be converted back to GSH by glutathione reductase (GR). Glutathione S-transferase (GST) participates in the detoxification of xenobiotic compounds by catalyzing their conjugation with GSH to form nontoxic products. Under normal physiological conditions, GR maintains more than 98% of intracellular glutathione in a reduced form, helping to maintain the intracellular environment in the reduced state. In situations where the redox system is intact, there is recovery of GSH, but under conditions of excess oxidising agents and/or failure of the protective system, it is assumed that there is an imbalance between GSH consumption and GSSG production (i.e., deficit in GPx) or an imbalance between GSSG consumption and GSH production (i.e., deficit in GR) (12).

Isoenzymes are preferentially expressed in specific tissues and have differing substrate affinity. GPx1 is the most abundant intracellular isoenzyme and GPx3 predominates in plasma.

Glutathione peroxidases are implicated in MS pathogenesis and for example GPx1 is upregulated in MS plaques. EAE rat model studies showed elevation of GPx3 in CSF compared with controls **(13).**

Interestingly, GSH as other thiols may interact with nitric oxide (_NO) to neutralize it and at the same time providing additional regulatory mechanism for ROS-related processes like s-nitrosylation **(5).** This pathway not only decreases _NO level, but also creates buffer for this gaseous signal transmitter and provides its transportation on relatively long distances and protects thiol groups from irreversible oxidation during oxidative boots **(14).**

Glutathione imbalance and or depletion has been reported to be involved in many brain disorders such as autism, Alzheimer's disease, Parkinson's disease, bipolar disorder, schizophrenia, amyotrophic lateral sclerosis, Huntington's disease, cerebrovascular syndrome, lumbar cervical discopathy, amyotrophic lateral sclerosis, Guillain-Barre´syndrome and multiple sclerosis. GSH deficit may precede the neuropathology of these diseases and neuronal GSH depletion may be a primary cause of these brain diseases **(15).**

Glutathione has been reported to protect mitochondrial complex I activity against nitrosative stress as S-nitrosoglutathione is formed, when this complex increases its content of nitrotyrosine and nitrosothiol groups in response to nitrosative stress, its activity is inhibited and therefore ATP production is diminished, which causes neuronal degeneration. The ratio GSH/ GSSG (usually 10:1) is considered consistent with the concept of oxidative stress as an important in the pathogenesis and progression of MS. Moreover, Although the decrease in GSH alone is not responsible for the degeneration of glial cells and neurons, reduced GSH could increase the susceptibility to other stressful factors which contribute to neuronal damage at glia and neuron cells **(3).**

Mechanisms of axonal damage are the consequence of the presence of TNF-α, matrix metallo-proteinases (MMPs), ROS, antibodies, increased glutamate, and aspartate and these molecules cause excitotoxicity in MS patients **(3).** Glutamate is increased in MS patients (active lesions) especially in white matter of normal appearance. Mature oligodendrocytes and astrocytes are highly sensitive to glutamate due to the expression of AMPA and NMDA receptors. Increased ROS by activated microglia (specialized macrophages of the CNS) during the immune response gives a state of increased lipid peroxidation and the oligodendrocyte is the most susceptible cell to be damaged by ROS **(16).**

Magnetic resonance specteroscoy in MS patients revealed reduced GSH levels compared with controls, decrease in GSH concentration in lesions of white matter in MS patients and showed a statistically significant decrease in GSH concentration in the grey matter of MS patients compared to controls. Concentration of GSH in the frontoparietal region in MS shows a tendency to decrease in patients with greater disability, as evaluated by EDSS **(11).**

Biochemical analysis of post mortem brains has provided evidence for the generation of oxidative stress during the course of the disease since the total GSH content is reduced by 40–50% compared to controls. Also in several brain regions, we have found increased levels of lipid peroxidation **(17).**

Oxidative stress increases between patients with clinically isolated syndrome (CIS) and patients with RRMS and is correlated with higher EDSS scores, lesion load, and disease duration in RRMS. GPx activity was lower in RRMS patients than in CIS patients and lower in CIS patients than in healthy controls. GPx activity was lower in SPMS patients than in healthy controls **(18).**

Oxidative stress and antioxidants have been the subject of intense research in order to prevent, reduce or even reverse the manifestations of MS. **Acrolein** (2-propenal) plays an important role in oxidative stress in many diseases including neurological disorders. It is an unsaturated aldehyde found in various endogenous and exogenous sources. It commonly appears as an environmental pollutant released from the cigarette manufacturing process, exhausts from combustion fumes and superheated cooking oil. It is also produced endogenously by oxidation of various compounds. This substance is toxic to neuronal tissue, catalysing the production of ROS, and is found in high concentrations in patients with MS. Acrolein is the most reactive lipid peroxidation (LPO) product, consuming GSH 100–150 times faster than 4-hydroxynonenal, another unsaturated aldehyde, thereby stimulating the production of ROS. In addition, acrolein readily forms conjugates with proteins and GSH (GS propionaldehyde) that remain highly reactive and have a half life much longer than free acrolein. This reaction is essential for their elimination although it decreases GSH reserves, limiting the ability of the organism to react against additional oxidative stress. Thus, there is strong evidence that this substance plays a critical role in oxidative stress, as demonstrated by its long half-life, its ability to generate ROS and its potent cytotoxicity. Hydralazine is an anti-hypertensive agent which binds and neutralizes acrolein and acrolein-binding protein, forming a compound that reduces the cellular toxicity of acrolein and permits safe excretion **(19).**

Melatonin (N-acetyl-5-methoxy- tryptamine) has antioxidant activity, its health benefits like optimization of blood pressure**,** potential beneficial effects on retinopathy in diabetic rats**,** it can improve glucose metabolism via correction of insulin production, protecting pancreatic b-cells against ROS-induced damage. Interestingly, last years a capability of melatonin to regulate expression of certain genes via specific regulatory pathways was disclosed**.** It increases erythrocyte SOD activity and erythrocyte GPx activity in SPMS patients with the dose of 10 mg daily for 30 days. It also decreases in erythrocyte membrane lipid peroxidation in SPMS patients which is particularly significant as there are currently no treatments available for SPMS **(20).**

The direct potential neuroprotective effects of **dimethyl fumarate** (DMF) and its primary metabolite monomethyl fumarate (MMF) was investigated by in work on cellular resistance to oxidative damage in primary cultures of CNS cells, which showed that treatment with DMF or MMF increased the redox potential cellular levels of glutathione, ATP and mitochondrial

membrane potential. similarly, **pegoretti et al., (1)** found that it stimulates nuclear factorerythroid2-related factor (Nrf2) which bind to DNA sequence called Antioxidant Response Element (ARE) to detoxify dangerous metabolites. The clinical usefulness of DMF as oral therapy (BG-12) in MS is being explored through phase III trials.

lipoic acid (LA), an essential co-factor for the conversion of pyruvate to acetyl-CoA, a critical step in cellular respiration. Together with its reduced form, dihydrolipoic acid (DHLA), LA reduces and recycles cellular antioxidants such as glutathione and acts as an eliminator of ROS and RNS, and modulates signal transduction pathways. These functions suggest that LA can be therapeutically effective in the treatment of MS **(21).**

N-acetylcysteine (NAC)

Although direct administration of GSH with the aim to increase GSH in the brain has not been successful because oral administration of GSH results in its rapid degradation in the gut and if given intravenously, GSH is rapidly oxidized to GSSG. Instead, N-acetylcysteine (NAC), liposomes encapsulated with GSH and Whey protein supplement are preferred as potential therapeutic agents to replenish GSH stores **(22).**

N-acetyl derivative of the amino acidL-cysteine (NAC) is rapidly absorbed following oral administration and it can cross the BBB, after absorption it is rapidly metabolized to cysteine, which is a direct precursor of GSH. It also regulates the glutamatergic, neurotrophic, and inflammatory pathways. Under the pro-oxidant condition of the brain, L-cysteine can get oxidized to cystine, which is the substrate of the cystine-glutamate antiporter that increases glutamate release into the extracellular space in exchange for cystine. Inside the cell, cystine can be reduced to cysteine which is used for GSH synthesis. NAC has been in use for more than 30 years for the treatment of acetaminophen-induced hepatotoxicity, its main side-effects are nausea, vomiting, and diarrhea. Infrequently anaphylactic reactions were observed due to the histamine release **(22).**

Liposomes encapsulated with GSH are also being developed for neuroprotection and to facilitate the delivery of GSH across the BBB. These are made of lecithin and glycerol and they get hydrolyzed to release GSH after crossing the BBB. The advantages of using these liposomes are low toxicity, low immune reaction, and high ability to cross the BBB **(23)**

Whey protein supplement (Immunocal) may be a novel therapeutic agent in neurodegenerative diseases. It acts by increasing the available GSH pool and mitigating damage by oxidative stress at a dose of 10–45 g/day for 2 weeks to 6 months has been shown to increase the GSH levels. It is rich in sulfur-containing amino acids such as methionine and cysteine, and cysteine is the precursor of GSH. The oral administration of whey protein supplement can enhance the concentration of GSH in neurons. It is likely well tolerated when used appropriately, but high doses can cause some side-effects such as increased bowel movements, nausea, thirst, bloating, cramps, reduced appetite, fatigue, and headache **(22).**

Omega-3 poly unsaturated fatty acids (PUFAs)

The mechanism of action for omega-3 PUFAs is suggested to be attributed to immune modulation and antioxidant action, omega-3 PUFAs decrease the production of inflammatory mediators (eicosanoids, cytokines, and ROS) and the expression of adhesion molecules. They both act directly by replacing amino acid (AA) as an eicosanoid substrate and inhibiting AA metabolism and indirectly by altering the expression of inflammatory genes through effects on transcription factor activation. Omega-3 PUFAs also give rise to anti-inflammatory mediators (resolvins and protectins) . Effects of resolvins and protectins include reducing neutrophil trafficking, cytokine, and ROS regulation and lowering the magnitude of the inflammatory response **(24)**

In evaluating the effect of consumption of omega-3 PUFAs on some markers of oxidative stress at the peripheral level, findings showed the decrease in serum levels of TNFα, IL-1β, IL-6, and NO metabolites compared with the placebo group **(25).**

The brain tissue, with a considerable number of phospholipid membranes, is very sensitive to the action of radicals due to a significant presence of mitochondria and consequently massive oxygen metabolic processes. A number of studies document the participation of OS in MS pathophysiology. Oxidative stress processes participate in both inflammatory and neurodegenerative pathophysiological components of MS. Oxidative stress is associated with the dysregulation of axonal bioenergetics, cytokine-induced synaptic hyperexcitability, abnormal iron accumulation, and the oxidant/antioxidant balance. Markers of OS assessed in the serum, erythrocytes, CSF, saliva, and urine may have diagnostic properties whereas antioxidants may have clinical application in the future. There are at least a couple of OS markers of the disease course which can be particularly useful in the diagnosis of severe forms of MS such as SPMS and PPMS. Other useful applications include markers of relapse and OS markers of disability. There might be some new hope in an objective assessment of the severity of MS.

Many antioxidants may have a positive impact on the course of MS. These substances include melatonin, dihydroasparagusic acid, n-3 polyunsaturated fatty acid (PUFA), α- (alpha-) lipoic acid (ALA) and others (including plant origin antioxidants). Innovative therapies are aimed in particular at neuroprotection and neurodegeneration. Potential drugs include compounds such as hydralazine, exendin-4, glucagon-like peptide-1 (GLP-1), and also lovastatin which protects against OS-induced cell death by the expression of PGC-1α and Nrf2.

Currently, a number of new studies focus on the immunotherapy and OS. Natalizumab and fingolimod have a positive effect on antioxidant capacity and may result in a reduction in OS markers. The relationship between DMF and OS is best known and is associated with the modulation of OS molecules. On the other hand, mitoxantrone is a drug that may be responsible for an increase in OS. There are new suggestions combining mitoxantrone therapy with

antioxidant supplementation such as N-acetylcysteine and melatonin in order to alleviate the toxicity of mitoxantrone.

Summarizing, using OS markers as biomarkers of MS severity or relapse could be a long-awaited helpful diagnostic tool. Moreover, adding antioxidants to immunotherapy which is well-established in MS may be reasonable and highly beneficial for MS patients due to their ability to reduce OS. Further research should be done to test new antioxidants for their effectiveness.

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